

# COMPARATIVE TOXICITY OF ANABASINE AND NICOTINE SULPHATES TO INSECTS<sup>1</sup>

By JOSEPH M. GINSBURG, *biochemist in entomology*, JOHN B. SCHMITT, and PHILIP GRANETT, *research assistants*, New Jersey Agricultural Experiment Station

## HISTORICAL REVIEW

Within the last few years the Government of the Union of Soviet Socialistic Republics has placed anabesine sulphate on the American market as a new contact poison selling at a price considerably lower than nicotine sulphate. Anabesine is an alkaloid present in the stems and leaves of *Anabasis aphylla* L., a member of the Chenopodiaceae. It is somewhat similar to nicotine in its structural formula and has the same empirical formula ( $C_{10}H_{14}N_2$ ). Anabesine is considered an isomer (7)<sup>2</sup> of nicotine. Like nicotine, it can be readily extracted from the plant and converted into a sulphate.

*Aphylla* is only one of a large number of species belonging to the genus *Anabasis*. It is a perennial weed growing (10) in northern Africa, Russia, Armenia, and in the neighboring countries. Attempts are at present being made to cultivate it on the North American continent. The amount of alkaloid varies from a fraction of 1 percent in old twigs and thick leaves to more than 2 percent in young succulent twigs and young growing leaves. The commercial product anabesine sulphate contains approximately 40 percent of total alkaloids, of which about 70 percent is anabesine (1, 7), the remainder being lupinine, other higher alkaloids, and miscellaneous plant material.

It may be of interest that not long before Orechhoff and Menschikoff (8) had isolated anabesine from the plant, Smith (9) synthesized, in 1930, the alkaloid  $\beta$ -pyridyl- $\alpha$ -piperidine, which he called "neonicotine" and which he later (11) found to be chemically the same as the natural anabesine. The only difference between the two is that neonicotine is optically inactive while anabesine is levorotatory. Smith, Richardson, and Shepard (12) found neonicotine as toxic to *Aphis rumicis* L. as nicotine.

Garman (2, 3, 4) reports that both anabesine and anabesine sulphate are more toxic to aphids than are nicotine and nicotine sulphate. In his experiments, dilutions of 1 pint of either anabesine sulphate or nicotine sulphate to 100 gallons of water produced equally high control of the white apple leafhopper. On the other hand, Campbell, Sullivan, and Smith (1) found anabesine less toxic than nicotine to culicine mosquito larvae.

<sup>1</sup> Received for publication May 17, 1935; issued October 1935. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Department of Entomology.

<sup>2</sup> Reference is made by number (italic) to Literature Cited, p. 354.

## EXPERIMENTAL WORK

In May 1931 the senior writer tested the toxicity to honeybees of anabesine sulphate received from the Amtorg Trading Corporation. Applied as a contact spray, concentrations of 0.2 and 0.1 percent killed 100 percent of the bees within 24 hours. When the properties of anabesine, as an internal poison, were tested by allowing the bees to feed on honey containing 0.2 percent of anabesine sulphate, only about 10 percent died within the same period. On the other hand, in a previous publication (5) the senior writer reported high percentages of kill of bees fed on honey containing 1 part of nicotine, in the form of nicotine oleate, to 3,200 parts of honey mixture. These preliminary results suggested that anabesine is much more effective as a contact poison than as a stomach poison.

Experiments with anabesine sulphate were resumed during the summer of 1934. Since nicotine is largely used in agricultural sprays to control aphids, the major part of this investigation was devoted to comparing the aphicidal properties of the two insecticides. The samples used in these tests contained 40.5 percent and 40 percent total alkaloids for nicotine sulphate and anabesine sulphate, respectively, as stated in the chemical analysis submitted by the manufacturers.

The tests with various dilutions of anabesine sulphate and nicotine sulphate were conducted on several species of aphids, on silk moth larvae, and on grasshoppers. The toxicity to insects was determined by methods previously described (6); 90 percent kill or higher was considered efficient control.

## TESTS ON APHIDS

The following species of aphids were used in these tests: *Aphis pomi* De G., on apple; *Aphis rumicis*, Lin. on nasturtium; *Macrosiphum rosae* Lin. on roses; *Macrosiphoniella sanborni* Gill and *Rhopalosiphum rufomaculata* Wils., on chrysanthemum. In order to spread and wet efficiently, each spray solution had added to it 0.2 percent of coconut-oil soap. Several series of tests were run for each dilution. The average results are reported in the tables.

## LABORATORY TESTS

Check tests with the wetting agent alone have shown that 0.2 percent of soap killed approximately 14 percent of the green apple aphids (*Aphis pomi*), 21 percent of the nasturtium aphids (*A. rumicis*), and 13 percent of *Macrosiphum rosae*. The concentrations of the spray mixtures ranged from 1 pint to one twenty-fourth of a pint per 100 gallons. The high dilutions, although not practical, were necessary in order to evaluate the differences between the toxicity of the two alkaloids. The results from tests on the aphids are set forth in table 1. A comparison of the results with apple and nasturtium aphids reveals no distinct differences in toxicity of the two insecticides in concentrations of one-half or one-third of a pint per 100 gallons, the percentage of kill being 90 or better in each case. At concentrations of one-sixth and one-twelfth of a pint the results were

decidedly higher, on both species of aphids, with anabesine sulphate than with nicotine sulphate. The rose aphids were more resistant to both anabesine and nicotine, 1 pint of anabesine sulphate being required per 100 gallons to produce about a 90 percent kill; the percentages of dead rose aphids were consistently higher with anabesine sulphate than with nicotine sulphate.

TABLE 1.—*Toxicity tests with anabesine and with nicotine sulphates on green-apple aphids (Aphis pomi), nasturtium aphids (A. rumicis), and rose aphids (Macrosiphum rosae)*

Insecticide in 100 gallons of spray <sup>1</sup> (pints)	Green-apple aphids		Nasturtium aphids		Rose aphids	
	Total insects	Dead after 24 hours	Total insects	Dead after 24 hours	Total insects	Dead after 24 hours
Anabesine sulphate:	<i>Number</i>	<i>Percent</i>	<i>Number</i>	<i>Percent</i>	<i>Number</i>	<i>Percent</i>
1.....					321	90.3
$\frac{1}{2}$ .....	888	94.8			436	78.4
$\frac{1}{3}$ .....	1,342	97.6	511	95.8	334	66.0
$\frac{1}{6}$ .....	618	93.4	322	90.9	608	60.0
$\frac{1}{12}$ .....	1,098	66.4	447	78.0		
$\frac{1}{24}$ .....	745	31.0	370	53.7		
Nicotine sulphate:						
1.....					372	87.6
$\frac{1}{2}$ .....	1,200	97.3			336	59.0
$\frac{1}{3}$ .....	1,736	90.2	272	92.6	471	52.4
$\frac{1}{6}$ .....	435	82.3	272	86.0	383	48.5
$\frac{1}{12}$ .....	1,982	58.9	788	66.7		
$\frac{1}{24}$ .....	686	38.1	273	48.7		
Check, 0.2 percent of soap: 0.....	1,338	14.7	716	21.4	301	13.0

<sup>1</sup> A concentration of 1 pint of insecticide per 100 gallons of spray represents an approximate dilution of the alkaloids of 1 to 2,000.

#### GREENHOUSE TESTS

Several beds of chrysanthemum plants, in a commercial greenhouse, were divided into two parts and sprayed with the two insecticides on August 2, 1934. In each case only one concentration, namely, one-third of a pint of insecticide per 100 gallons of spray solution, was used. The plants were infested with two different species of aphids, the black (*Macrosiphoniella sanborni*) and the green (*Rhopalosiphum rufomaculata*). After approximately 24 hours, gross observations showed a practically complete kill of the black aphids and an incomplete kill of the green aphids with each of the spray mixtures. In order more closely to verify these differences, several plants were removed from each plot, and counts of live and dead aphids were made. The results, set forth in table 2, show 100-percent control of black aphids with both spray mixtures. The control of green aphids disclosed striking differences in favor of the anabesine sulphate, the actual percentages killed being 87.8 for anabesine sulphate and 32.1 for nicotine sulphate. The experiments were repeated on August 8 on different chrysanthemum plants in the same greenhouse. Only the green aphids were counted. The results (table 2) again show high kill with anabesine sulphate and low kill with nicotine sulphate. It appears, therefore, from these results that anabesine possesses higher toxicity to this species of aphids than does nicotine.

TABLE 2.—*Toxicity tests with anabesine and with nicotine sulphates*<sup>1</sup> on two species of aphids, *Macrosiphoniella sanborni* and *Rhopalosiphum rufomaculata*, infesting chrysanthemum plants

Insecticide tested	Date of spraying	Black aphids, <i>Macrosiphoniella sanborni</i>		Green aphids, <i>Rhopalosiphum rufomaculata</i>	
		Total insects on 4 plants	Dead after 24 hours	Total insects on 4 plants	Dead after 24 hours
		Number	Percent	Number	Percent
Anabesine sulphate.....	Aug. 2, 1934	297	100	296	87.8
Nicotine sulphate.....	do.	173	100	205	32.1
Anabesine sulphate.....	Aug. 8, 1934			31	87.1
Nicotine sulphate.....	do.			329	28.8

<sup>1</sup> One-third pint of insecticide per 100 gallons of spray.

### TESTS ON CHEWING INSECTS

#### LABORATORY TESTS ON SILK MOTH LARVAE

The silk moth larva (*Bombyx mori* L.) has proved to be a satisfactory insect-indicator for testing stomach poisons in this laboratory. A comparison of the toxicity of anabesine and nicotine sulphates to healthy silk moth caterpillars was, therefore, made. Because of the scarcity of the insects at the time of testing, only 10 insects, all in the fourth instar, were used for each single test. In order to eliminate volatility of the alkaloids, 0.1 percent of a neutral wetting agent<sup>3</sup> was added to each spray mixture, instead of soap to produce efficient spreading.

The results, presented in table 3 show that concentrations of 1 quart of nicotine sulphate to 100 gallons of water gave a 100-percent kill in 2 days; whereas anabesine sulphate gave a 30-percent kill in 3 days. In concentrations of 1 pint to 100 gallons, nicotine sulphate gave about 95-percent kill and anabesine sulphate only 15-percent kill in 3 days.

TABLE 3.—*Toxicity tests with anabesine and with nicotine sulphates on silk-moth larvae, using 10 larvae per test*

Insecticide tested	Insecticide per 100 gallons of spray <sup>1</sup>	Dead—		
		After 1 day	After 2 days	After 3 days
	Pints	Percent	Percent	Percent
Nicotine sulphate.....	2	90	100	
Do.....	1	40	90	<sup>2</sup> 100
Do.....	1	30	70	<sup>2</sup> 90
Anabesine sulphate.....	2	0	10	<sup>3</sup> 30
Do.....	1	0	0	<sup>3</sup> 10
Do.....	1	0	10	<sup>3</sup> 20
Check (0.1 percent of arecap).....	0	0	0	0
Do.....	0	0	0	0

<sup>1</sup> See note, table 1, for approximate dilution of the alkaloids.

<sup>2</sup> Average, 95.

<sup>3</sup> Average, 15.

<sup>4</sup> Arecap obtained from Monsanto Chemical Co., St. Louis, Mo.

## LABORATORY TESTS ON GRASSHOPPERS

Adults of a short-horned grasshopper (*Melanoplus femur-rubrum*, De G.), commonly called "red-legged locust", collected from a meadow near the college campus, were transferred to cages and fed on young potted tomato plants, sprayed 1 hour previously, with various concentrations of nicotine sulphate and anabesine sulphate. Twenty insects were used for each test. Each spray solution contained 0.1 percent of the neutral wetting agent (Arescap). The tomato plant is evidently very palatable to this species of grasshopper, since the insects rapidly devoured the leaves on the check plants, sprayed only with 0.1 percent of Arescap, so that the plants had to be renewed once during the 3 days of testing. The feeding on the plants sprayed with the two insecticides, however, was rapidly retarded after the first day especially on those sprayed with nicotine sulphate, and in neither case was the renewal of plants necessary.

The results, presented in table 4, show that nicotine sulphate is decidedly more toxic to this chewing insect than is anabesine sulphate. At concentrations of 1 pint and 1 quart per 100 gallons of spray, nicotine sulphate produced 80- and 90-percent kill, respectively, as compared with 40- and 60-percent mortality obtained with the same concentrations of anabesine sulphate.

TABLE 4.—*Toxicity tests with anabesine and with nicotine sulphates on grasshoppers, in which 20 insects per test were used*

Insecticide tested	Insecticide per 100 gallons of spray	Dead after 3 days
	<i>Pints</i>	<i>Percent</i>
Nicotine sulphate.....	1	80
Do.....	2	90
Anabesine sulphate.....	1	40
Do.....	2	60
Check, 0.1 percent of Arescap.....	0	15

## SUMMARY AND CONCLUSIONS

Laboratory and greenhouse tests were conducted with anabesine sulphate and nicotine sulphate on several species of aphids, silk moth larvae, grasshoppers, and honeybees. The results definitely show that:

Anabesine sulphate equals or excels nicotine sulphate in toxicity to *Aphis rumicis*, *A. pomi*, and *Macrosiphoniella sanborni* and is decidedly more toxic to *Rhopalosiphum rufomaculata* and *Macrosiphum rosae*.

Anabesine sulphate possesses very little toxicity as a stomach poison against silk moth larvae, while nicotine sulphate proved highly toxic to this insect.

Anabesine sulphate was decidedly less toxic to grasshoppers, applied as a stomach poison, than was nicotine sulphate.

## LITERATURE CITED

- (1) CAMPBELL, F. L., SULLIVAN, W. N., and SMITH, C. R.  
1933. THE RELATIVE TOXICITY OF NICOTINE, ANABASINE, METHYL ANABASINE, AND LUPININE FOR CULICINE MOSQUITO LARVAE. *Jour. Econ. Ent.* 26: 500-509. illus.
- (2) GARMAN, P.  
1933. NOTES OF THE COMPARATIVE TOXICITY OF ANABASINE SULFATE AND NICOTINE SULFATE FOR APHIS AND LEAFHOPPERS. *Conn. State Agr. Expt. Sta. Bull.* 349: 433-434.
- (3) ———  
1934. STUDY OF APHICIDES. *Conn. State Agr. Expt. Sta. Bull.* 360: 458-461, illus.
- (4) ——— and TOWNSEND, J. F.  
1934. CONTROL OF THE WHITE APPLE LEAFHOPPER, 1933. *Conn. State Agr. Expt. Sta. Bull.* 360: 449-451.
- (5) GINSBURG, J. M.  
1928. INSECTICIDE INVESTIGATIONS. *N. J. Agr. Expt. Sta. Rept.* (1927-28) 49: 158-163.
- (6) ——— SCHMITT, J. B., and GRANETT, P.  
1934. DERRIS INSECTICIDES: I. TOXICITY OF VARIOUS EXTRACTS OF DERRIS ROOT TO SUCKING AND CHEWING INSECTS. *N. J. Agr. Expt. Sta. Bull.* 576: [3]-16.
- (7) NELSON, O. A.  
1934. SOME PHYSICAL CONSTANTS OF ANABASINE. *Jour. Amer. Chem. Soc.* 56: 1989-1990.
- (8) ORECHOFF, A., and MENSCHIKOFF, F.  
1931. ÜBER DIE ALKALOIDE VON ANABASIS APHYLLA L. (I. MITTEIL.) *Ber. Deut. Chem. Gesell.* 64: 266-274.
- (9) SMITH, C. R.  
1931. NEONICOTINE AND ISOMERIC PYRIDYLPYPERIDINES. *Jour. Amer. Chem. Soc.* 53: 277-283.
- (10) ———  
1931. NEONICOTINE RECENTLY FOUND AS AN ALKALOID IN ANABASIS APHYLLA L. (*Sci. Note*) *Jour. Econ. Ent.* 24: 1108.
- (11) ———  
1932. IDENTITY OF NEONICOTINE AND THE ALKALOID ANABASINE. *Jour. Amer. Chem. Soc.* 54: 397-399.
- (12) ——— RICHARDSON, C. H., and SHEPARD, H. H.  
1930. NEONICOTINE AND CERTAIN OTHER DERIVATIVES OF THE DIPYRIDYLS AS INSECTICIDES. *Jour. Econ. Ent.* 23: 863-867.